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RESPONSES OF FORAGE GRASSES TO ALUMINUM IN SOLUTION CULTURE

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ABSTRACT

Growth, nutrient uptake and nutrient uptake efficiency differences in orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb), and timothy (*Phleum pratense* L.) were evaluated at 0, 100, 200, and 300 µM Al. In each of the species, cultivar differences were also compared. In the absence of Al stress, cultivars of orchardgrass outperformed other grasses. The presence of Al reduced shoot and root growth; however, the magnitude of the growth reduction depended upon the species and cultivars. The growth of shoots and roots showed a significant difference with respect to species, cultivars, treatment Al and their interactions. Aluminum reduced the uptake of many essential nutrients. At 100 µM Al Potomac orchardgrass had the highest and climax timothy had the lowest mineral content. The efficiency ratio (ER) assisted in classifying grass entries into efficient and inefficient utilizers of the absorbed nutrients. The ER is defined as milligrams of dry shoot weight produced per milligram of element in the shoot. The ER for P, K, Cu and Zn gave a positive correlation with shoot weight; however, in general, negative relationships were observed for shoot growth and ER for Mg, Fe, and Mn. In all the species increasing Al concentration from 0 to 100 µM increased ER for Mg and decreased ER for K and Zn. With the exception of tall fescue cultivars, the ER for P was reduced by 100 µM Al. The species and cultivars used in this study showed inter- and intraspecific differences in growth, uptake, and ER for nutrients in the presence or absence of Al stress. Significant reduction in growth, even at 100 µM Al by all the three species of grass indicates that these grass species are far more sensitive to Al than the field crops. Therefore, experiments with levels of Al lesser than 100 µM would have given a better outlook on the performances of these grass species.

INTRODUCTION

In many acid soils Al toxicity is probably the most important growth limiting factor for plants (6). The presence of toxic levels of Al and deficient levels of P, Ca, and Mg are major factors for low forage production on many acid soils of the Appalachian region. Plant species and cultivars within species are known to differ widely in their tolerance to aluminum. Varietal and genotypic differences in tolerance to soil acidity have been observed for fescues (12,13), weeping lovegrass (8), bermudagrass (11), and Kentucky bluegrass (12). In these studies toxicity symptoms on leaves and dry matter of shoots and roots have been used as criteria in differentiating Al stress tolerance among cultivars or genotypes. Tall fescue, orchardgrass and timothy are cool season grasses adapted to the temperate regions of the world; however, their adaptibility to acid soils is still unknown. Inter- and intraspecific differences of plant growth and mineral composition in the presence or absence of Al have been well documented (1,2,3,5,8,10,11,12). In many species and cultivars within species, Al tolerance is related to nutrient uptake, transport and use efficiency (5,6). Cultivars with a high nutrient efficiency ratio under Al stress may have an advantage in adapting to the mineral stress ecosystem of Appalachia.

In the current study three cool season grass species, tall fescue, orchardgrass and timothy were tested for their responses to Al under climatically controlled conditions. Two cultivars were used for each of the species. The objectives were to evaluate shoot and root growth, mineral uptake and mineral efficiency ratios in grass species after treatment with various levels of Al in nutrient solution culture.

MATERIALS AND METHODS

Seedlings of orchardgrass (*Dactylis glomerata* L., cultivars Hallmark and Potomac), tall fescue (*Festuca arundinacea* Schreb, cultivars Forager and KY-31), and timothy (*Phleum pratense* L., cultivars Clair and Climax) were raised in perlite medium under mist chambers in a greenhouse. Eighteen day old seedlings were suspended into nutrient solution which was held in 14 liter polyethylene tubs. High density styrofoam was used as a top to hold plants in place. The 1/5 strength Steinberg solution similar to the one proposed by Foy et al. (7) was used. Four levels of Al (0, 100, 200, and 300 μM) were established by adding Al as $Al_2(SO_4)_3 \cdot 18H_2O$. Solution pH was adjusted initially to 4.5 and left unadjusted thereafter. Water with an adjusted pH of 4.5 was added to the tubs to maintain the solution levels. During growth the nutrient solution was continuously aerated. The experiment was carried out in a climatically controlled growth room with light intensity of 530 µmole M^{-2} s^{-1}, imposed for 14 hours daily. The relative humidity was maintained at 60%. Temprature of the growth room was 28°C in the light cycle and 22°C in the dark cycle. A complete randomized design with three replications was used.

The experiment was terminated after 34 days of growth in nutrient solution. At harvest, plant shoots and roots were separated, washed in deionized water, dried at 70°C for 3 days, and dry weights were recorded. Plant samples were ground to pass through a 0.55 mm mesh sieve and digested in a H_2O_2:H_2SO_4 mixture. Elemental determinations were made by inductively coupled plasma emission spectroscopy (ICP).

The relative growth reduction (RGR) for both shoots and roots, the efficiency ratio (ER) and percent inhibition of nutrient uptake (PI) were calculated as follows: (1,9)

RGR = [1-(Growth with Al/Growth without Al)] X 100 [1]

ER = Milligrams of dry shoot wt/milligram of element in shoot [2]

PI = $[(U_0 - U_1)/U_0]$ X 100 [3]

where U_0 and U_1 refers to the total content of any given mineral element in the shoot at 0 and 100 µM Al, respectively.

The forage grass species and their cultivars used in this study showed a greater degree of separation at 100 µM Al; with respect to growth of shoots and roots; therefore, this level of Al was chosen for comparison of nutrient uptake, inhibition and ER.

RESULTS AND DISCUSSION

Shoot and root growth

Dry matter accumulation and relative growth reduction (RGR) of shoot and roots at various Al levels are given in Table 1. In the absence of Al, both cultivars of orchardgrass outperformed

TABLE 1

Growth and relative growth reduction (RGR) for shoot and root of grasses at various Al levels.

Special Cultivar	Growth 0 μM Al		Relative Growth Reduction (RGR) 100 μM Al		200 μM Al		300 μM Al	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	----g/10 plants----		----------%----------					
<u>Orchardgrass</u>								
Hallmark	9.64	2.99	91	72	94	94	97	98
Potomac	8.16	2.95	67	69	88	86	96	96
<u>Tall Fescue</u>								
Forager	6.68	2.50	78	77	92	93	95	97
KY-31	3.00	1.30	71	79	82	86	94	97
<u>Timothy</u>								
Clair	3.88	1.95	65	79	90	95	95	97
Climax	0.79	0.79	42	73	92	96	89	96

<u>Analysis of Variance - F Value</u>

Source of Variance	Shoot Wt.	Root Wt.	RGR Shoot	RGR Root
Species (S)	45.0**	26.7**	0.56NS	0.08NS
Cultivars (C)	9.1**	7.2**	0.36NS	0.05NS
Treatment Al (T-Al)	184.1**	149.8**	52.73**	64.73**
S X T-Al	25.5**	8.8**	0.42NS	0.07NS
C X T-Al	6.1**	3.8**	0.22NS	0.04NS
<u>Among T-Al</u>				
Linear	424.4**	354.4**	128.48**	151.91**
Quadratic	115.4**	87.1**	28.32**	38.78**
Cubic	12.5**	8.0**	0.24NS	3.49NS

*,**Significant at 0.05 and 0.01 levels of probability, respectively.
NS = Not Significant.

other entries. Among entries, Climax timothy gave the least dry matter accumulations in both shoots and roots.

Aluminum in the growth medium reduced shoot and root dry matter. Based on RGR at 100 µM Al, differences among entries was more than 2-fold for shoot weight and more than 1-fold for root weight. At this level, Hallmark orchardgrass recorded the largest reduction in shoot weight (91%) and Climax timothy recorded the lowest reduction in shoot weight (42%) as compared to control. Based on RGR of shoot at 100 µM Al, Forager tall fescue and Hallmark orchardgrass are very sensitive to Al and Climax timothy is moderately tolerant to Al. Fleming et al. (4) reported that in a solution culture of pH 4, the 148 µM Al severely inhibited shoot and root growth of tall fescue, however, Al concentration of 445 µM were required to bring similar growth reduction of lovegrass. Murry and Foy (12) have reported the performances of 6 tall fescue cultivars in limed and unlimed Tatum acid soil. Relative top yield (pH 4.5/pH 4.7) of the tall fescue cultivars ranged from 1.8% for Fawn to 35.4% for Kentucky-31. In their study, tall fescue cultivars were much more tolerant to soil acidity than Kentucky bluegrass cultivars. In the current study, root weight of any given species was highly correlated with shoot weight. The RGR of shoots and roots was negatively correlated with shoot weight (Table 2). Dry weight and RGR of shoots and roots are the reliable traits for differentiating the magnitude of Al sensitivity among grass species. Using these traits, Baligar et al. (1) were able to separate red clover cultivars into Al tolerant and intolerant types.

Growth of shoots and roots showed highly significant differences with respect to species, varieties, Al levels, and their interactions (Table 1). The RGR for shoot and roots showed significant differences to Al levels only. Treatment Al levels were subdivided into linear, quadratic and cubic

TABLE 2

Correlation coefficients (r) relating shoot weight of orchardgrass, tall fescue and timothy to root growth, RGR for shoot and root, nutrient uptake and ER.

Variables	Orchardgrass	Tall Fescue	Timothy
Root wt.	0.98**	0.99**	0.98**
RGR - Root	-0.98**	-0.89**	-0.70**
RGR - Shoot	-0.99**	-0.88**	-0.68**
U P	0.97**	0.99**	0.96**
U K	0.80**	0.95**	0.91**
U Mg	0.97**	0.97**	0.96**
U Cu	0.90**	0.97**	0.97**
U Fe	0.97**	0.98**	0.92**
U Mn	0.99**	0.99**	0.98**
U Zn	0.91**	0.86**	0.96**
ER - P	0.71**	0.38NS	0.61**
ER - K	0.94**	0.90**	0.68**
ER - Mg	-0.88**	-0.73**	-0.48**
ER - Cu	0.47*	0.55**	0.67**
ER - Fe	-0.25NS	-0.39NS	0.20NS
ER - Mn	-0.24NS	0.63**	-0.11NS
ER - Zn	0.66**	0.55**	0.43*

*, ** Significant at 0.05 and 0.01 levels of probability, respectively
NS = Not Significant.

components to investigate the various responses of shoots and root weights and RGR for Al treatments. Significant linear and quadratic responses were observed to the Al treatment for shoot and root growth and RGR.

TABLE 3

Nutrient uptake (U) and percent inhibition of uptake (PI) at 100 μM Al in different grass species/cultivars.

Element	Orchardgrass Hallmark	Orchardgrass Potomac	Tall Fescue Forager	Tall Fescue KY-31	Timothy Clair	Timothy Climax
			Uptake (U)†			
P	0.7	2.3	1.0	0.6	1.1	0.4
K	13.7	46.7	15.5	9.0	17.1	5.8
Mg	0.5	1.6	1.3	0.8	1.0	0.4
Cu	5.5	31.2	10.5	5.1	9.2	4.1
Fe	32.4	144.8	46.5	28.7	95.0	22.4
Mn	27.0	114.1	71.2	32.9	39.7	15.8
Zn	1.6	6.4	3.5	2.4	3.6	1.3
			Percent Inhibition (PI)			
P	88	60	80	70	58	-24
K	73	-21	50	42	19	-97
Mg	97	89	91	88	87	66
Cu	93	19	69	62	58	25
Fe	92	55	80	70	30	31
Mn	94	71	67	68	73	38
Zn	91	52	71	52	50	-2

†U = mg•10 plant^{-1} for P, K, and Mg; μg•10 plant^{-1} for Cu, Fe, Mn and Zn.

Uptake and Inhibition of Nutrient Uptake

Since the growth of all the grass species was considerably poor at 200 and 300 µM Al, comparisons of nutrient uptake and percent inhibition (PI) of nutrients were made at the 100 µM Al (Table 3). Potomac orchardgrass was the most efficient and Climax timothy was the most inefficient in uptake of nutrients. This is reflected in higher or lower shoot weight in these cultivars. In any given species, uptake of nutrients was highly correlated with shoot weight (Table 2). Plant demand for any given nutrient is a functon of its growth rate and internal ionic concentrations (14,16).

Reduction in shoot growth due to the presence of Al in growth medium is responsible for the reduced nutrient uptake. Aluminum is also known to interfere with the uptake, transport and use of several essential elements (1,2,4,5,6). In the current study, 100 µM Al reduced nutrient uptake of various elements by 19 to 91% relative to the control (Table 3). However, in presence of 100 µM Al, Climax timothy increased uptake of P, K, and Zn and Potomac orchardgrass increased the uptake of K. In general, timothy cultivars experienced least reduction in element uptake by Al. Baligar et al. (1) also noted the enhanced uptake of K by some red clover cultivars subjected to 50 µM Al.

In the various grasses tested, significant differences in uptake of P, K, Mg, Fe and Mn were noted for species, cultivars and treatment Al and their interactions (Table 4). Species and treatment Al gave significant differences for Zn uptake. Aluminum treatment versus uptake for all elements had highly significant linear components. With the exception of K and Zn uptake, a significant quadratic and cubic relationship was observed for other elemental uptake and treatment Al.

Efficiency Ratio (ER)

The computed ER values assisted Baligar et al. (1) in differentiating red clover cultivars into efficient and

TABLE 4

Analysis of variance (F value) for treatment and interaction effects on nutrient uptake and efficiency ratios in various grass species and cultivars.

Source of Variation	P	K	Mg	Cu	Fe	Mn	Zn
				Uptake			
Species (S)	48.6**	51.2**	17.4**	43.4**	38.5**	48.4**	7.6**
Cultivars (C)	12.8**	10.8**	6.6**	3.4*	4.6**	3.9*	1.3NS
Treat Al (T-Al)	132.4**	51.8**	167.3**	80.4**	112.1**	119.8**	33.6**
S X T-Al	15.8**	6.0**	14.6**	15.5**	18.8**	27.5**	3.3**
C X T-Al	4.6**	5.7**	5.3**	11.4**	3.8**	2.2*	1.0NS
T-Al							
Linear	314.7**	154.5**	332.4**	198.8**	275.4**	269.6**	80.4**
Quadratic	57.9**	0.7NS	146.0**	37.6**	52.9**	76.2**	18.0**
Cubic	24.7**	0.1NS	23.6**	4.8*	7.9**	13.4**	2.3NS
				Efficiency Ratio (ER)			
Species (S)	12.8**	41.7**	102.8**	33.6**	529.4**	26.6**	1.6NS
Cultivars (C)	2.5NS	3.2*	25.6**	14.0**	348.4**	13.0**	3.3*
Treat-Al (T-Al)	34.3**	689.5**	206.7**	37.0**	1498.2**	19.8**	16.8**
S X T-Al	2.2NS	9.7**	7.9**	3.1*	319.4**	13.8**	0.4NS
C X T-Al	0.6NS	1.9*	10.1**	8.1**	303.0**	1.9NS	0.2NS
T-Al							
Linear	61.8**	1244.4**	479.8**	96.1**	2587.6**	7.0*	47.4**
Quadratic	15.4**	803.4**	138.1**	11.3**	1571.5**	3.6NS	2.6NS
Cubic	25.8**	20.7**	2.1NS	3.6NS	335.6**	48.7**	0.6NS

*,**Significant at 0.05 and 0.01 levels of probability, respectively.
NS = Not Significant.

inefficient utilizers of the absorbed nutrients either in presence or absence of Al. Such an evaluation of species and cultivars within species appears to be especially important under acid soil where supply of essential nutrients is very much limited. The cultivars with a high ER value for essential nutrients might be able to perform well in acidic infertile soils. The ER for K, Cu, and Zn in the various grass species

TABLE 5

Efficiency ratios (ER)† for nutrients in shoots of grass species/cultivars at 0 and 100 μM Al treatment.

Element	Orchardgrass		Tall Fescue		Timothy	
	Hallmark	Potomac	Forager	KY-31	Clair	Climax
			0 μM Aluminum			
P	1737E§	1416	1516	1462	1433	1370I
K	193	205	213E	182	177	166I
Mg	680E	538	448	435I	497	450
Cu	116	204	198	211I	182	100I
Fe	23	24	30E	26	30E	15I
Mn	21	19	31E	28	26	18I
Zn	610	609	631E	483	556	440I
			100 μM Aluminum			
P	1270	1269	1552E	1520	1229	1060I
K	63	57I	96E	95	79	78
Mg	1940E	1811	1172	1097I	1375	1239
Cu	159	92I	141	170E	156	119
Fe	27	19	32E	30	16I	20
Mn	31	24	21I	26	35E	29
Zn	555E	420	456	357I	442	400

†ER for Cu, Fe, Mn and Zn need to bemultiplied by 10^3.

§E = Most efficient; I = Most inefficient.

gave positive significant relationships with shoot dry weight (Table 2). With a few exceptions, the ER for Mg, Fe and Mn in various grass species gave a significant negative relationship with shoot dry weight.

The ER for various elements in grass species and cultivars at 0 and 100 µM Al are shown in Table 5. In all species, increasing Al from 0 to 100 µM increased ER for Mg and decreased ER for K and Zn. With the exception of tall fescue cultivars, the ER for P was reduced due to the presence of 100 µM Al. However, the magnitude of ER for Cu, Fe and Mn appears to be dependent upon species, cultivars and their response to Al levels.

In absence of Al, Hallmark orchardgrass was the most efficient utilizer of absorbed P and Mg. However, Forager tall fescue was the most efficient utilizer of absorbed K, Fe, Mn and Zn. The Climax timothy was the most inefficient utilizer of absorbed P, K, Cu, Fe, Mn and Zn. At 100 µM Al Hallmark orchardgrass was most efficient utilizer of absorbed Mg and Zn and Forager tall fescue was most efficient ultilizer of P, K, and Fe. From the present results it appears that genetic diversity does exist in these grass species for nutrient uptake efficiency. The inter- and intraspecific differences in mineral uptake and utilization in various crops in presence or absence of Al is well documented (1,2,3,9,10,15).

Significant differences in ER for K, Mg, Cu, Fe and Mn were noted for Al treatment species, cultivars and their interactions. The ER for P gave significant differences for species treatment Al. A significant linear and quadratic response for Al was observed for the ER values of P, K, Mg, Cu, and Fe.

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